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Short Communications

Antibacterial activity of *Helichrysum pedunculatum* callus cultures

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Helichrysum pedunculatum (Asteraceae) is used by the Xhosas of the Transkei in South Africa to dress wounds after circumcision. As a verification of its folkloric use, the plant extract from this herb was earlier investigated for its antibacterial activity. A callus culture of this species was established in half strength Murashige and Skoog medium enriched with naphthalene acetic acid and kinetin. The homogenised dichloromethane extract of the callus was evaluated for its antibacterial activity by direct bioautography on TLC. The extract inhibited the growth of the Gram-positive bacteria, *Bacillus cereus, B. pumilus, B. subtilis* and *Staphylococcus aureus* as well as the Gram-negative bacterium, *Serratia marcescens*.

Keywords: Antibacterial; callus; *Helichrysum pedunculatum*; tissue culture; traditional medicine.

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The antimicrobial properties of a number of *Helichrysum* (Asteraceae) species have been described (Dekker *et al.* 1983; Boily & Van Puyvelde 1986; Tomas-Barberan *et al.* 1990; Rios & Villar 1991; Meyer & Afolayan 1995; Meyer & Dilika 1996). A South African species, *Helichrysum aureonitens* showed antibacterial activity against *Micrococcus kristinae, Bacillus cereus, B. pumilus* and *Staphylococcus aureus* (Meyer & Afolayan 1995). Although work has been done on the antimicrobial activity of *Helichrysum* species, very little has been published on callus induction and the testing of these cultures for antibacterial activity. Leeuwner and Meyer (1995) induced callus from *H. aureonitens* and showed that it was active against *B. subtilis, S. aureus, Klebsiella pneumoniae* and *Escherichia coli*.

Helichrysum pedunculatum Hilliard and Burrt has been reported in folklore to have medicinal value. These include its ability to cure stomach ailments and its anti-inflammatory activity (Watt & Breyer-Brandwijk 1962; Hilliard 1983; Bolofo & Johnson 1988). The plant is used by Xhosas in Transkei (South Africa), to dress wounds especially after circumcision. The claimed medicinal property of the plant was substantiated by Meyer and Dilika (1996) who reported the antibacterial activity of the extract against *B. cereus, B. subtilis, B. pumilus, M. kristinae, S. aureus, Enterobacter cloacae* and *Serratia marcescens*.

In this paper, we describe the establishment of *H. pedunculatum* callus cultures and present the results of their antibacterial properties against ten bacterial species.

Callus induction was achieved by surface sterilising young leaves from *H. pedunculatum* for 30 seconds in an aqueous solution of 0.35% sodium hypochlorite (NaOCl) followed by rinsing 5 times in sterile distilled water. Sterile leaf material was then excised into 5 × 5 mm pieces and placed on half strength Murashige and Skoog (1962) (MS) basal medium (ICN Biomedicals Inc., UK) supplemented with 3% sucrose, 1 mg/l naphthalene acetic acid (NAA) and 0.05 mg/l kinetin. The medium was solidified with 0.5% agar (Biolab, SA).

Homogenous calli appeared within three weeks (25°C, 24 h day length; light intensity 35.5 µmol m^{-2} s^{-1}) and were maintained by sub-culturing every six weeks onto fresh medium as described above but solidified with 0.3% agar. The soft friable callus was spread cautiously on the surface of the medium. The spreading was necessary to secure a sufficiently large area for secondary metabolite production which is reported to be generally restricted to the upper cell layers (Dodds & Roberts 1986). Callus cultures were maintained in light at 25°C and sub-cultured on fresh MS medium every 6 weeks. The antibacterial bioassay was performed after five transfers to fresh medium.

Callus material was agitated in cold water in order to remove the growth medium from the tissue without damaging the callus, dried and weighed (6.885g). Metabolites were extracted by homogenising the callus in dichloromethane (CH_2Cl_2). The homogenate was filtered and the sediment re-extracted by stirring three times in CH_2Cl_2 for 30 minutes each. The filtrate was concentrated to dryness under reduced pressure, yielding 39 mg residue. The sample was re-dissolved in 1 ml CH_2Cl_2.

The antibacterial activity of the callus extract was tested against the following Gram-positive bacterial species: *B. cereus, B. pumilus, B. subtilis, M. kristinae, S. aureus* and the Gram-negative species: *E. cloacae, E. coli, K. pnuemoniae, P. aeruginosa* and *S. marcescens*. Each organism was maintained on nutrient agar (Biolab, SA) and recovered for testing by growth in nutrient broth (No. 2, Biolab, SA) for 24 hours at 37°C except *P. aeruginosa* which was harvested only after 72 hours.

The antibacterial activity of the callus extract was tested by direct bioautography on thin layer chromatography (TLC) plates. The callus extract (156 µg from a 39 mg/ml solution) was applied to silica gel 60 plates (Merck, Germany). An extract of *H. pedunculatum* leaves (Meyer & Dilika 1996) was also applied to the same TLC plate. The plate was developed in benzene:chloroform (40:60 v:v). It was observed under UV light (254 and 366 nm) after development, left overnight for the solvents to evaporate completely and sprayed with the bacterial suspensions.

The bacterial suspensions were obtained as follows; 24 h (and 72 h *P. aeruginosa*) old bacterial cultures in nutrient broth were centrifuged at 3000 rpm for 20 min. The supernatant was discarded and the sedimented bacteria re-suspended in fresh nutrient broth to an absorbance of 0.84 at 560 nm (Lund & Lyon, 1975). The bacterial suspensions were then sprayed with a fine spray onto the TLC plates and the plates dried for a few minutes until they appeared transluscent. The plates were then incubated at 25°C for 24 h (and 72 h for *P. aeruginosa*) in humid conditions whereafter they were sprayed with an aqueous solution of 2.0 mg/ml *p*-iodonitrotetrazolium violet and reincubated at 25°C for 3 to 72 h depending on the bacterial species. Any inhibition of bacterial growth could clearly be seen as white spots on a red background.

More compounds were produced by the plant than the callus culture when observed on TLC plates. This confirms the findings by Das and Law (1990), Thorpe (1990) and Missaleva *et al.* (1993), that secondary metabolites in a source plant may not be present in its callus.

E. coli, P. aeruginosa, K. pneumoniae, M. kristinae and *E. cloacae* showed resistance against all the compounds of the callus extract. Certain compounds in the callus extract were, however, active against *B. cereus, B. pumilus, B. subtilis, S. aureus* and *S. marcescens*. When the plant extract was tested with the agar dilution method (Meyer & Dilika 1996), all the above mentioned Gram-positive bacteria were inhibited as well as the Gram-negative bacteria, *S. marcescens, E. cloacae* and *M. kristinae*. The higher activity found in the agar dilution method is probably due to the effect of the combined antibacterial compounds in the extract when tested in Petri-dishes rather than individual compounds being tested after being chromatographed on a TLC plate.

Although not all the compounds produced by the plant were found in the callus extract, both extracts contained compounds that had more activity on Gram-positive bacteria than on Gram-negative ones. These results are consistent with other studies which also showed that plant extracts inhibit Gram-positive bacteria more than Gram-negative ones (Grosvenor *et al.* 1995; Meyer & Dilika 1996).

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In vitro culture of *Mondia whitei* (Periplocaceae), a threatened Zulu medicinal plant

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Single-node explants of *Mondia whitei* (Hook.f.) Skeels derived from *in vitro* cultured seedlings were used to produce rooted plantlets on the medium of Murashige and Skoog (1962) supplemented with 1 mgl^{-1} BA, both in the absence and presence of charcoal, and solidified with 0.3% w/v Gelrite. Eighty five percent of the plants were successfully hardened off under a 20/4 h light/dark photoperiod and conditions of 80–100% humidity. *M. whitei* is a highly prized and consequently over-exploited Zulu medicinal plant which is destructively harvested for its strongly aromatic roots. These are used for both their medicinal and food spice attributes. This micropropagation protocol allows for *ca.* 2000 plantlets to be produced from a single seed following 7 to 8 subcultures at 4 to 6 week intervals.

Keywords: conservation, micropropagation, *Mondia whitei*, muthi, tissue culture, Zulu medicinal plant.

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Mondia whitei (Hook.f.) Skeels is the more widespread of the two species comprising the genus *Mondia* Skeels (Periplocaceae), both of which are lianas endemic to South-Central and East Africa (Bullock 1961). At the time of its discovery and subsequent description by taxonomists the over-exploitation of *Mondia* by the Zulu was clearly evident (Hooker 1871). By 1915, Medley-Wood already considered *M. whitei* to be one of the first medicinal taxa likely to become locally extinct. The popularity in Natal of *M. whitei* a century ago was chronicled by Bryant (1909): 'Every native fortunate enough to procure (some) habitually carries about with him a supply...of the root and chews the same whenever the digestion may seek relief'. Although its conservation status was recently listed by Hilton-Taylor (1996) as 'Vulnerable' in KwaZulu-Natal, the global status was given as 'not threatened'. A status re-evaluation may result following consideration of a report (Bouquet 1970 in Neuwinger 1994) which describes comparable unsustainable exploitation in tropical Africa, particularly in the Congo. In KwaZulu-Natal, *Mondia* was once known widely from coastal and midlands forests; it is currently considered extinct in the wild to the south of the Tugela river, and in Zululand is largely restricted to protected coastal swamp forests. Its popularity may stem from the pleasant aromatic character and taste of the rootstock (Bryant 1909); from the roots an isomer of vanillin has been isolated (Goulding & Pelly 1911). Besides acting as a tonic (Medley-Wood & Evans 1899) and settling the stomach (Hooker 1871), the recorded medicinal uses of the root have variously included the easing of flatulence (Gerstner 1941) abdominal pains and constipation, even the treatment of bilharzia (Gelfand *et al.* 1985). When mixed with *Tiliacora chrysobotrya* Welw. the combination is reported to form a mild laxative as well as a chest remedy (De Ficalho 1947). From Zimbabwe northwards to East-Central